CENTER FOR URBAN WATERS
UNIVERSITY of WASHINGTON | TACOMA

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TO: Brad Roberts,
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Environmental Protection Division
Washington State Attorney General's Office

FROM: Dr. Edward Kolodziej, Ph.D.
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RE: Chemical Analysis of Crumb Rubber Samples-Electron Dam Site
Case Name: Electron Hydro LLC; [10906767]

Summary of Findings

Center for Urban Waters Analytical Team

I, Edward Kolodziej, am currently employed as an associate professor by the University of Washington. I was hired by the U. of Washington in 2014 as part of a "Freshwater Sciences" cluster hire. As an associate professor, I split my time between research and teaching duties and have maintained substantial research activity in environmental analytical chemistry throughout my career. My faculty appointment is split between the U. of Washington-Tacoma (67%, Division of Sciences and Mathematics) and U. of Washington-Seattle (33%, Department of Civil and Environmental Engineering); I am tenured and teach and conduct research activities on both UW campuses. I also am a Principal Investigator at the Center for Urban Waters (Tacoma, WA) which is an off-campus research facility affiliated and administratively managed by the U. of Washington-Tacoma.

My primary technical and research expertise is water quality assessment, particularly with respect to currently unregulated organic contaminants, including compounds such as pharmaceuticals, personal care products, and industrial chemicals. I especially work at the intersection of water, chemicals, and fish, seeking to identify, track, and treat chemicals that are harmful to fish or other aquatic organisms. To accomplish this type of research, I have operated and worked within environmental mass spectrometry laboratories throughout my entire academic career (22+ years), focused on the detection, identification, and quantification of organic contaminants in water and other sample types. I have over 18 years of experience working on diffuse "non-point" pollution systems such as agricultural and urban runoff and stormwater.

Over the past 7 years, my primary research activities have focused on investigating the chemical linkages between urban stormwaters and related observations of acute mortality in coho salmon. During the course of these investigations, we deeply investigated the role of roadway runoff and tire rubbers in water quality degradation, including extensive extraction and

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leaching of tire rubbers with water and organic solvents to understand its chemical composition and potential for environmental pollution. Ultimately, we linked observations of coho mortality primarily to a previously unknown, yet globally ubiquitous, tire rubber derived chemical "6PPD-quinone" (Tian et al. *Science*, 2021). Currently, my research group at the Center for Urban Waters would generally be considered to be one of the global leaders in understanding the impacts of tire rubbers and roadway runoff on water quality. Our larger collaborative team, including the Washington Stormwater Center (Washington State U.-Puyallup and the Center for Urban Waters), NOAA-National Marine Fisheries Service, and the U.S. Fish and Wildflife Service, would also generally be considered a global leader in understanding the impacts of urban stormwaters on salmonids and other aquatic organisms.

Dr. Zhenyu Tian, Ph.D., assisted these analytical efforts by performing sample characterization, sample processing, and some data analysis. Dr. Tian worked at the Center for Urban Waters in my (EPK) research group as a research scientist and post-doctoral scholar from February 2018 to August 2021. Dr. Tian is now a faculty member in the Department of Chemistry and Chemical Biology at Northeastern University. Dr. Tian has worked for over 7 years in environmental mass spectrometry and is already considered a global leader in the use of high resolution mass spectrometry for water quality analysis. Dr. Tian discovered 6PPD-quinone and first linked its formation to ozonation of the antioxidant parent chemical 6PPD.

Investigation Overview

In early 2021, I was contacted by the WA Attorney General's office regarding the artificial turf and crumb rubber spill event/s that occurred at the Electron Hydro facility on the Puyallup River from July-October 2020. These spill events introduced a substantial amount of both artificial turf material and embedded crumb rubber materials into the Puyallup River. Over spring 2021, I worked with the WA Attorney General's office, the Washington Department of Fish and Wildlife, and the EPA National Enforcement Investigations Center to discuss the spill events and develop a sampling plan to acquire crumb rubber and turf material samples for chemical analysis. The primary concern of these efforts was the potential ability of the crumb rubber spill events to introduce harmful chemical and metal pollutants into the Puyallup River.

In particular, a key discussion question revolved around the common use of recycled tire rubbers in turf based crumb rubber materials and the potential contributing role of these materials to water pollution of the Puyallup River. Over the past 4 years, our research efforts have been demonstrating the high specific lethality of tire rubber leachates (contacting water with bits of tire tread) to coho salmon, including the isolation and identification of 6PPD-quinone from tire rubber leachates that were consistently and repeatably toxic to coho salmon at environmentally relevant concentrations. Our data indicated that 6PPD-quinone was both ubiquitous in tire rubber leachates, roadway runoff, and was the "primary causal toxicant" behind widespread observations of stormwater-linked acute mortality events in coho salmon. Therefore, exposure to tire-rubber derived 6PPD-quinone in particular posed an extremely high risk to salmonid health, although it certainly is not the only harmful chemical or constituent expected for tire rubbers and materials derived from tire rubbers. Our data, and others, has demonstrated the substantial impairment of water quality arising from various other chemicals present in tire rubber leachates, although these chemicals were not ultimately specifically linked to acute mortality events in coho salmon.

Field Sampling

Following the developed sampling plan (attached), three locations related to the Electron Hydro LLC spill event were sampled on April 1-2, 2021 by EPA NEIC staff and the WA Department of Fish and Wildlife. After storage in an evidence locker at a Washington Department of Fish and Wildlife facility, samples were brought by the EPA NEIC staff to the Center for Urban Waters (Tacoma, WA) on May 17, 2021, and split by EPA NEIC into A and B samples for concurrent defense analysis.

Samples were collected from three locations: 1) the "Quarry", which represented the source storage area where the artificial turf materials brought to the Electron Dam site originated; 2) the "Intake", located in close proximity to the diversion dam site, and where materials removed from the diversion dam location were stored; and 3) the "Forebay" where additional artificial turf materials, including those directly removed from the river, were stored. Samples generally consisted of 2.5 L amber glass jars filled with artificial turf (green or colored artificial grass and woven backing materials) and/or rubber infill materials (small fine bits of rubber, mostly black, some colored white, roughly mm in scale). Visual inspection revealed no broken containers or other issues with sample integrity or label clarity.

Crumb rubber infill materials contained fine quartz type sands for drainage and volume purposes in addition to the rubber grains (Figure 1). Provided turf materials also had some residual crumb rubbers and sand enmeshed in the rubberized woven sublayer and artificial grass material. Much of the "turf" sample (by composition) was comprised of the stiff, rubberized woven sublayer material. This sublayer supported the overlaying artificial grass and infill materials (Figure 2). Typically, it was difficult to fully remove the residual crumb rubbers from the turf sublayers, the grass and woven materials easily trapped crumb rubber infill materials. While Figure 1 demonstrates what was extracted for analysis, Figure 2 also demonstrates the residual crumb rubber and sand materials that could be shaken out of the turf samples upon handling and shaking.

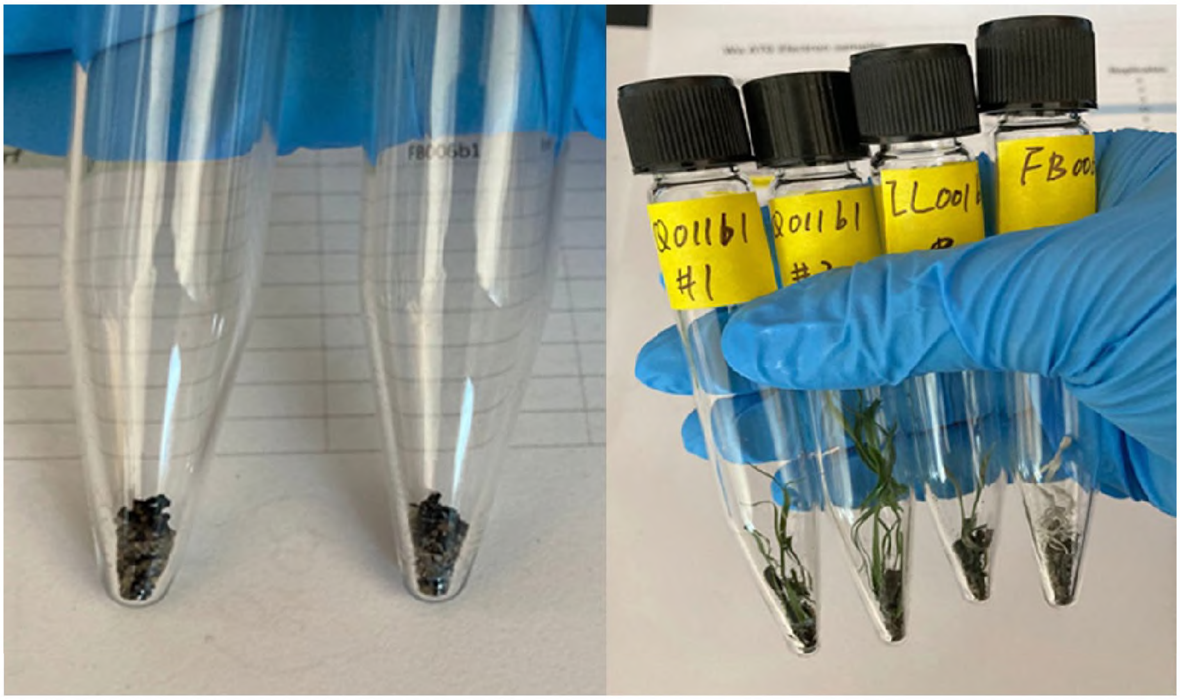

Figure 1: Crumb rubber infill sample (left) showing mixture of crumb rubber and fine sand. On right, turf sample showing mixture of artificial grass, rubberized sublayer, and mixed crumb rubber that was subsequently solvent extracted and analyzed.

Figure 2. Turf material sample Q015b1 as a representative turf sample. Left panel shows the quantity of crumb rubber and sand shaken out of the turf grass after loose shaking, and is representative of those combined materials solvent extracted (including grass, sublayer, and loose infill) for 6PPD-quinone analysis. Right panel shows the rubberized woven sublayer which composed most turf material mass.

Table 1: Sample labels and descriptions of all Electron Hydro LLC samples brought to CUW by the EPA NEIC team on 5/17/2021. "a" samples represent crumb rubbers; "b" samples represent artificial turf materials with crumb rubber briefly shaken out. We note that "b" samples typically contained some crumb rubber and sand stuck to the artificial turfs, or caught in the rubberized woven sublayer which could not be easily separated or removed.

Sample ID	Description	Location
Q010 a1	Turf Roll #1-Crumb Rubber	Quarry
Q010 b1	Turf Roll #1-Turf	Quarry
Q011 a1	Turf Roll #3-Crumb Rubber	Quarry
Q011 b1	Turf Roll #3-Turf	Quarry
Q012 a1	Turf Roll #2-Crumb Rubber	Quarry
Q012 b1	Turf Roll #2-Turf	Quarry
Q013 a1	Turf Roll #6-Crumb Rubber	Quarry
Q013 b1	Turf Roll #6-Turf	Quarry
Q014 a1	Turf Roll #5-Crumb Rubber	Quarry
Q014 b1	Turf Roll #5-Turf	Quarry
Q015 a1	Turf Roll #4-Crumb Rubber	Quarry
Q015 b1	Turf Roll #4-Turf	Quarry
IL001 a1	Turf Roll #1-Crumb Rubber	Intake
IL001 b1	Turf Roll #1-Turf	Intake
IL002 a1	Turf Roll #2-Crumb Rubber	Intake
IL002 b1	Turf Roll #2-Turf	Intake
IL003 a1	Loose crumb rubber from handling pile at Intake	Intake
FB006 a1	Turf Roll #1-Crumb Rubber	Forebay
FB006 b1	Turf Roll #1-Turf	Forebay
FB007 a1	Turf Roll #2-Crumb Rubber	Forebay
FB007 b1	Turf Roll #2-Turf	Forebay
FB008 a1	Loose crumb rubber from handling pile at Forebay	Forebay
Quality Assurance-Quality Control Samples		
S004	Used nitrile gloves-Intake	
S005	Clean nitrile gloves for background	
S009	Used nitrile gloves-Forebay	
S016	Used nitrile gloves-Quarry	

Laboratory Analysis

Noting visual differences in some of the sample compositions, the above samples were first characterized for crumb rubber density and physical composition on May 18, 2021. After shaking and homogenizing, sub-samples were removed from large sample jar using precleaned stainless steel tweezers and small stainless steel spatulas by Dr. Kolodziej and Dr. Tian. After a number of preliminary trials, initial sorting efforts manually separated sand particles from rubber particles on weighing paper, accumulating sufficient mass of each sample type to attain a density-mass relationship for both organic rubber materials ("Rubber" in Table 2) and inorganic sand materials ("Sand" in Table 2). On May 25th, 2 mL samples were weighed to determine the approximate rubber-sand composition of the provided crumb rubber materials. Results are presented in Table 2.

Table 2: Density (of loose bulk material, incudes pore space) and estimated rubber-sand sample compositions of the provided crumb rubber samples. Average, median, and standard deviation data are also provided for the entire group of crumb rubber samples

Sample	Observed Density [g/mL]	%Rubber-Vol.	% Sand-Vol.	%Rubber-Mass	%Sand-Mass
Rubber	0.605	100	0	100	0
Sand	1.522	0	100	0	100
Q010a1	1.002	57	43	34	66
Q011a1	1.030	54	46	32	68
Q012a1	1.076	49	51	27	73
Q013a1	1.071	49	51	28	72
Q014a1	0.770	82	18	64	36
Q015a1	0.880	70	30	48	52
IL001a1	1.058	51	49	29	71
IL002a1	1.412	12	88	5	95
IL003a1	1.159	40	60	21	79
FB006a1	0.812	77	23	58	42
FB007a1	1.105	45	55	25	75
FB008a1	1.194	36	64	18	82
Average	*1.047*	*52*	*48*	*32*	*68*
Median	*1.064*	*50*	*50*	*28*	*72*
Stdev	*0.174*	*19*	*19*	*17*	*17*

Analytical Results

Samples were next analyzed for 6PPD-quinone using solvent extraction followed by liquid chromatography-tandem mass spectrometry using isotope dilution methodologies for quantification. This LC/MS/MS analytical method for 6PPD-quinone is currently awaiting accreditation by the Washington Department of Ecology; no current accredited method exists for 6PPD-quinone in Washington State by any laboratory. Similar LC/MS/MS analytical methods operated at the Center for Urban Waters have been accredited for >5 years currently. Briefly, 6PPD-quinone (10 mg, purity 98.8%, solid) and D5-6PPD-quinone (solution in acetonitrile, 100 mg/L) standards were purchased from HPC (Atlanta, GA, USA). Methanol (LCMS grade) and ethanol (absolute, 200 proof), and formic acid (HPLC grade) were purchased from Fisher Scientific. Deionized water (18 MΩ-*cm*) was generated by a MilliQ Ultrapure Water System. 6PPD-quinone stock solution was made by dissolving 5 mg solid into 50 mL ethanol, this stock was stored in a -20 °C freezer.

Sample processing and extraction was performed from June 22-24, 2021 by Dr. Tian. For each sample (unseparated mixture of sand and rubber), ~100 mg was weighed and transferred into a glass centrifuge tube. Turf samples were processed similarly, including artificial grass, rubberized sublayer, and residual crumb rubber and sand. 6PPD-quinone-d_5 (50 ng, 50 μL of 1 mg/L) was spiked onto the samples as the internal standard, and spiked samples were stored under room temperature overnight to allow for solvent evaporation. The samples were then extracted twice with 5 mL of methanol by vortexing (~1 min), shaking (rotary shaker, ~10 min, 60 rpm), and sonication (~20 min). The mixtures were centrifuged at 2500 rpm for 20 min, then the supernatants were combined and concentrated to 1 mL. The extracts were frozen at -20 °C overnight and filtered through a 0.2 μm PTFE syringe filter while still cold. The resulting filtrates were then split (100 µL from 1 mL) and diluted 10-fold with methanol for quantitative analysis on LC-MS/MS (to avoid detector saturation). The remaining undiluted filtrates (900 µL) were then analyzed on QTOF-HRMS for suspect and non-target screening.

Quality Assurance-Quality Control (QA/QC) plans for instrumental analysis were made upon consultation with EPA NEIC on June 17, 2021. For QA/QC, a method blank was made by passing pure LCMS grade methanol solvents through the same spiking/extraction procedure. Unused nitrile gloves taken to the field and provided by EPA staff were also extracted as part of the QA/QC process. Solvent blanks were injected along with the samples and other blanks. To monitor extraction performance and quantification accuracy, two samples (FB006a1 and IL001a1) were spiked with 6PPD-quinone (500 ng, 50 µL of 10 mg/L) along with the internal standard, and extracted with the same procedure.

Quantification of 6PPD-Q used an Agilent 1290 Infinity ultrahigh performance liquid chromatography (UHPLC) coupled to an Agilent 6460A triple quadrupole mass spectrometer (Santa Clara, CA, USA). Chromatography used a reverse phase C18 column (Agilent Poroshell HPH-C18 2.1 × 100 mm, 2.7 µm particle size) and C18 guard column at 45 °C, injection volume 5 µL, flow rate 0.2 mL/min, and binary gradient of 0.1% formic acid in each of water (A) and methanol (B): [50% B 0−0.5 min, 50%−100% B 0.5−10.5 min, 100% B 10.5−12 min, 100%−50% B 12−13 min; 50% B 13−15 min]. 6PPD-Q detection used electrospray ionization in positive polarity (ESI+) and multi reaction monitoring (MRM) mode. According to MS/MS data from HRMS and MRM screening, *m/z* 299→215 (14 eV) and 299→187 (26 eV) were selected as the qualitative and quantitative ion transitions. The 6PPD-Q concentrations were estimated from a seven-point calibration curve (0.025-50 µg/L) with D5-6PPD-Q as the isotopic internal standard (25 µg/L, same as in sample extracts) for response normalization; determination coefficients (R^2) were >0.995.

The LC/MS/MS instrument was regularly maintained and was last checked by an on-site Agilent technician on July 8, 2021. Performance was initially checked to assess detector sensitivity. Running calibration curves (Figure 3) indicated that the analyte response was linear, and was sensitive to the lowest point of the calibration curve with good signal to noise ratios even at the most dilute standard (0.025 ppb).

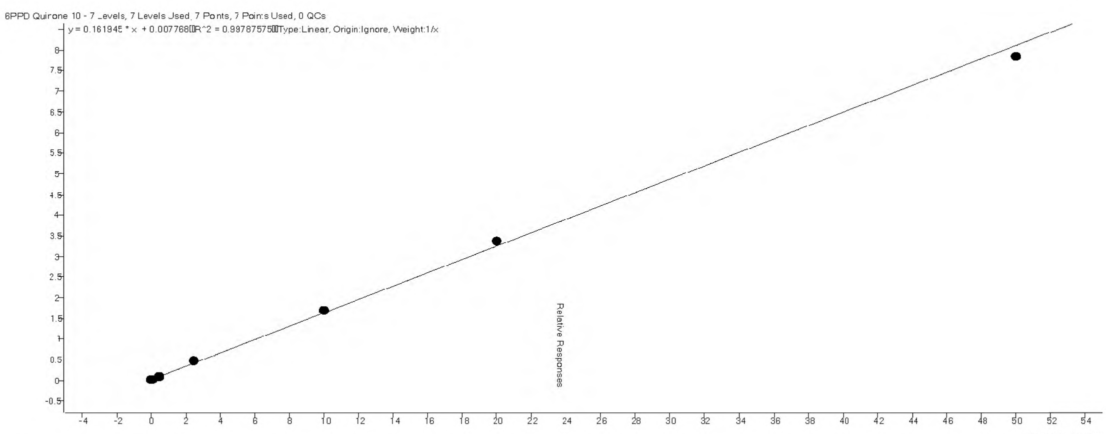

Figure 3. Seven-point calibration curve used to quantify 6PPD-quinone in crumb rubber samples by LC-MS/MS.

6PPD-quinone was quantified in the provided samples on August 2-3, 2021, by isotope dilution LC/MS/MS analysis. The isotopic internal standard response ranged from ~9500-16,000 (peak area count). Accuracy with respect to estimating concentrations of calibration samples ranged from 79-117%, indicating the calibration curve was effective. No peak carryover was observed in methanol blanks, and 6PPD-quinone retention times were consistent throughout the run (8.64-8.66 min), indicating stable and consistent chromatography and separation. Results of the 6PPD-quinone analysis by LC/MS/MS are presented in Table 3.

Table 3. 6PPD-quinone concentrations measured by LC/MS/MS in crumb rubber and turf samples (shaded blue) collected from Electron Hydro LLC locations. Average, median, and standard deviation data also are reported for the sample batch. QA/QC data are shaded green.

Sample ID	Description	Conc.-Mixture [μg/g mixture]	Conc.-Rubber [μg/g rubber]
Q010a1	Quarry-Turf Roll #1	1.0	3.1
Q012a1	Quarry-Turf Roll #2	1.0	3.7
Q011a1	Quarry-Turf Roll #3	0.9	2.7
Q011b1	Quarry-Turf Roll #3 Turf sample-Replicate 1	0.8	
Q011b1	Quarry-Turf Roll #3 Turf sample-Replicate 2	1.0	
Q015a1	Quarry-Turf Roll #4	0.3	0.6
Q014a1	Quarry-Turf Roll #5	0.7	1.0
Q013a1	Quarry-Turf Roll #6-Replicate 1	1.1	3.9
Q013a1	Quarry-Turf Roll #6-Replicate 2	0.9	3.4
IL001a1	Intake-Turf Roll #1-Replicate 1	1.1	3.7
IL001a1	Intake-Turf Roll #1-Replicate 2	0.7	2.5
IL011b1	Intake-Turf Roll #1 Turf sample	0.5	
IL002a1	Intake-Turf Roll #2-Replicate 1	0.2	3.9
IL002a1	Intake-Turf Roll #2-Replicate 2	0.03	0.7
FB006a1	Forebay-Turf Roll #1-Replicate 1	1.9	3.2
FB006a1	Forebay-Turf Roll #1-Replicate 2	1.3	2.3
FB006b1	Forebay-Turf Roll #1 Turf sample	0.8	
FB007a1	Forebay-Turf Roll #2-Replicate 1	0.8	3.1
FB007a1	Forebay-Turf Roll #2-Replicate 2	0.7	2.6
IL003a1	Intake-Loose Crumb Rubber Pile	0.4	2.1
FB008a1	Forebay-Loose Crumb Rubber	2.5	13.6
	Average (Crumb Rubber)	*0.91*	*3.3*
	Median (Crumb Rubber)	*0.85*	*3.1*
	Standard Deviation (Crumb Rubber)	*0.60*	*2.9*
	Average (Turf)	*0.75*	
	Median (Turf)	*0.76*	
	Standard Deviation (Turf)	*0.18*	
FB006a1	Forebay-Turf Roll #1 Spike	124% recovery	
IL001a1	Intake-Turf Roll #1 Spike	102% recovery	
	Solvent Blank (direct injection of solvent)	Not detected	
	Method Blank (solvent processed as a sample)	0.009 ppb	
S005	Sampling Gloves-Solvent Rinse	0.1 ppb	

To determine whether the chemical composition of the provided samples was consistent with waste tire rubbers as the source of the crumb rubber media, a subset of samples (extracts from all intake crumb rubber samples) also was analyzed by liquid chromatography-accurate mass time of flight mass spectrometry on August 18, 2021. Rather than focus on quantifying a small, user-defined group of analytes (e.g. as for LC/MS/MS analysis), this QTOF-HRMS analysis focuses on qualitative identification of chemicals detected in samples. Samples such as crumb rubber extracts typically contain hundreds to thousands of chemical detections as observed by QTOF-HRMS detection, with the vast majority of these remaining unidentified. High resolution mass spectrometry (HRMS) data are typically screened using "suspect screening" analyses where the observed accurate mass of all chemical detections is compared against user-generated lists of possible chemicals present in sample types such as crumb rubbers where some information as to their chemical composition and identity might be available.

Intake samples IL001a1, IL002a1, and IL003a1 were analyzed in triplicate using an Agilent 1290 Infinity ultrahigh performance liquid chromatograph (UHPLC) coupled to an Agilent 6530 Quadrupole Time-of-Flight high-resolution mass spectrometer (QTOF HRMS; Santa Clara, CA, USA). The QTOF HRMS instrument was regularly maintained and was last checked by an on-site Agilent technician on maintenance visits to the Center for Urban Waters from July 8-26, 2021. The instrument was tuned and mass calibrated prior to running samples, all parameters passed and were within expected and acceptable ranges. A reversed-phase C18 column (Agilent ZORBAX Eclipse Plus 2.1×100 mm, 1.8 µm) with a C18 guard column (2.1×5 mm, 1.8 µm) was used for the UHPLC separation at 45 °C with 5 µL injection volume. The peaks were separated with a gradient elution with mobile phases of 0.1% formic acid in each of deionized water (A) and methanol (B) as follows: 5% B at 0-1 min, 50% B at 4 min, 100% B at 17-20 min, 5% B at 20.1 min; stop time 22.5 min; post-time 2 min. The flow rate was 0.4 mL/min. Full scan data was acquired under 2 GHz Extended Dynamic Range mode at a range of 100-1700 m/z, MS/MS data were collected by data-dependent acquisition at the range of 50-1700 m/z, with collision-induced dissociation at 10, 20, and 40 eV. An internal standard control was analyzed every 6-8 samples. These suspect screening detections aligned with data from commercial standards, indicating high reporting confidence for these chemical identifications.

QTOF HRMS analysis indicated that in addition to 6PPD-quinone, the parent anti-oxidant compound 6PPD was present in extracts of all crumb rubber samples analyzed by QTOF-HRMS. Additionally, other compounds that we frequently detect in aqueous leachates of tire rubbers, or were reported within solvent extracts of crumb rubber samples (Peter et al. 2018, US EPA 2019) were present in extracts of all crumb rubber samples analyzed (see Appendix for chromatograms). Among the many QTPF-HRMS detections (358-1214 individual chemical detections across each of the 3 crumb rubber samples), 246 of these chemicals were detected in all three intake samples. While the vast majority of these chemical detections represent unknown compounds, these QTOF-HRMS detections included hexa(methoxymethyl)melamine (HMMM), used to bind steel belt within tire treads, diphenylguanidine (DPG), used as a vulcanization accelerator in rubber production, and several structurally related amines such as dicyclohexylurea, dicyclohexylamine, and the substituted diphenylamine antioxidant SDPA-C8C8 (Peter et al 2018, Hou et al 2019, Tian et al. 2021). In addition to the above detections, the compound bis(2-ethylhexyl)phthalate was present in all of these crumb rubber samples. This compound was frequently detected and reported by the EPA as an especially common chemical constituent of crumb rubbers (US EPA 2019), and is classified by the EPA as a "Group B2, Probable Human Carcinogen" with respect to its human health risk.

Summary and Implications

These environmental mass spectrometry data indicate that all of the crumb rubber samples analyzed from the quarry, intake, and forebay locations contained 6PPD-quinone. In addition to 6PPD-quinone, the samples also contained several other chemicals typical for tire rubbers such as 6PPD, diphenylguanidine, HMMM and other marker chemicals, indicating that these crumb rubber infills were likely derived from waste tires, or contained a large fraction of waste tire composition within the crumb rubber infills. These detections, along with the QTOF-HRMS data, also indicate that many synthetic chemicals were present in these crumb rubbers in addition to 6PPD-quinone and crumb rubbers are chemically complex.

The average concentration of 6PPD-quinone in the crumb rubber samples (mixture of rubber and sand materials) was 0.91 µg/g mixture, with a median concentration of 0.85±0.60 µg/g mixture. When normalized to the rubber content of the crumb rubbers (Table 2), the average 6PPD-quinone concentration was 3.3 µg/g rubber (median concentration 3.1±2.9 µg/g rubber) across all of the crumb rubber samples analyzed. For context, similar solvent extractions of tire tread particles derived from mixtures of new and used tire treads (e.g. Tian et al. 2021 or Peter et al 2018) generally yield 6PPD-quinone concentrations of ~10 µg/g rubber. The lower concentrations of 6PPD-quinone in these crumb rubbers is most consistent with weathering and the age of these Electron Dam crumb rubber samples, some of which were reported to have been in the quarry location for many years or even decades prior to their transport to the Electron Dam site. Detected concentrations in crumb rubbers were somewhat variable, ranging from a low of 0.56 µg/g rubber (Quarry roll #4) to a high of 13.6 µg/g rubber (loose crumb rubber from the forebay). Such variation may result from the different exposure conditions of some of these crumb rubbers, with top or edge rolls most exposed to weathering and environmental processes which tend to degrade organic contaminants and reduce concentrations. By contrast, interior rolls may have experienced more limited weathering and reduced chemical degradation, and thus reflect somewhat higher concentrations. Rainfall and exposure to water also would remove 6PPD-quinone, although it would continue to regenerate at rubber surfaces over time as air-exposed 6PPD in the tire rubbers oxidizes.

6PPD-quinone was also present in all of the turf samples analyzed, with an average concentration of 0.75 µg/g turf mixture. The presence of 6PPD-quinone is consistent with the rubberized sublayer composition, small amounts of residual crumb rubbers in the turf sublayer, and the presence of residual 6PPD-quinone absorbed into the sublayer and turf grass during the long period when these materials were in close physical contact. The detection of 6PPD-quinone in the turf materials indicates that the turf materials themselves were capable of acting as an additional source of some 6PPD-quinone in addition to contributions from the crumb rubbers.

Notably, the parent antioxidant chemical 6PPD was detected by QTOF-HRMS in all of the intake samples analyzed. While QTOF-HRMS analysis was not quantitative in this application, 6PPD was relatively abundant (large peaks detected) in these samples, indicating that a substantial mass of unreacted 6PPD still existed in these crumb rubbers. This is important because upon exposure to air and the trace levels of ground level ozone it contains, 6PPD would continue to react to form additional 6PPD-quinone and act as a continuous source of 6PPD-quinone until all 6PPD was completely depleted from the rubbers. Therefore, even as rain washes away 6PPD-quinone, or the river removed 6PPD-quinone mass from these materials, additional and continuing formation of 6PPD-quinone would be expected over time above and beyond the concentrations/mass directly detected by the LC/MS/MS analysis.

Our research experience with 6PPD-quinone indicates that it is moderately water soluble and easily capable of mobilizing from rubbers into water when wetted. For example, we have detected 6PPD-quinone in all roadway runoff and tire tread rubber leachate samples that we have analyzed (e.g. Tian et al 2021). Because 6PPD-quinone is water soluble and was present in all crumb rubber and turf samples analyzed, the Electron dam crumb rubber and turf spill events of July-October 2020 would have certainly contaminated the Puyallup River with 6PPD-quinone. Additionally, because crumb rubbers contain many chemicals, many other crumb rubber derived synthetic chemicals also would have been expected to pollute the river upon discharge, including chemicals like bis(2-ethylhexyl)phthalate with harmful attributes.

6PPD-quinone pollution of the Puyallup River in particular is of special concern because 6PPD-quinone is extremely toxic to coho salmon (Tian et al, 2021, McIntyre et al 2021). Our current estimate of its toxic potential is reflected by the measured “LC50” value (an exposure concentration which is acutely lethal to 50% of the exposed population over a 24 hour period) of 95 ng/L (i.e. 0.095 µg/L) for 6PPD-quinone. This extremely low LC50 value (lower values indicate more toxic) indicates that 6PPD-quinone is among the most acutely toxic compounds known to exist for fish. For comparison, compounds with LC50 values of 100 µg/L or lower for acute toxicity are classified as “very highly toxic” (the highest, most toxic, category) compounds by the US EPA. Among such “very highly toxic” compounds, only a very small group (<5) of organophosphate or organochlorine pesticides (e.g. parathion, mirex, guthion) are known to have similar or lower LC50 values for aquatic organisms than 6PPD-quinone does for coho salmon. While the toxicity of 6PPD-quinone has not been evaluated across many species to date (commercial standards only became available in spring 2021), the low LC50 value of 0.095 µg/L indicates that 6PPD-quinone exposure is extremely serious for coho salmon because extraordinarily small quantities can result in acute mortality.

Without direct sampling of the crumb rubber spill events of July-October 2020, the actual concentration of 6PPD-quinone (and any other crumb rubber or turf material derived chemicals) in the Puyallup River cannot be accurately known. Although such data does not exist, estimates of potential concentration and mass loadings can be derived from the measured concentrations of 6PPD-quinone in these materials and estimates of the quantity of crumb rubber and turf discharged to the river during the spill events. The report of Cherry (2020) noted “617 square yards (516 m^2) of field turf” were released into the river during the July 29-30, 2020 event. Using the median measured composition of crumb rubber infill mixtures (1.064 kg/L; Table 2) and the same assumptions reported by Cherry (617 yd^2, all crumb rubber infill material released, 0.75” of infill on the field turf) the spilled infill mixture would represent ~10,500 kg of crumb rubber and sand released to the river. Using the median 6PPD-quinone data (0.85±0.60 µg/g mixture) 8.89±6.22 g of 6PPD-quinone would have been discharged to the river during the spill. Additionally, the turf materials would contribute an additional 0.88±0.21 g of 6PPD-quinone, for an estimated total discharge of 9.77±6.43 g of 6PPD-quinone during the spill event under the above conditions. Figure 4 demonstrates similar calculations for various depths of infill on the 617 square yards of spilled field turf material.

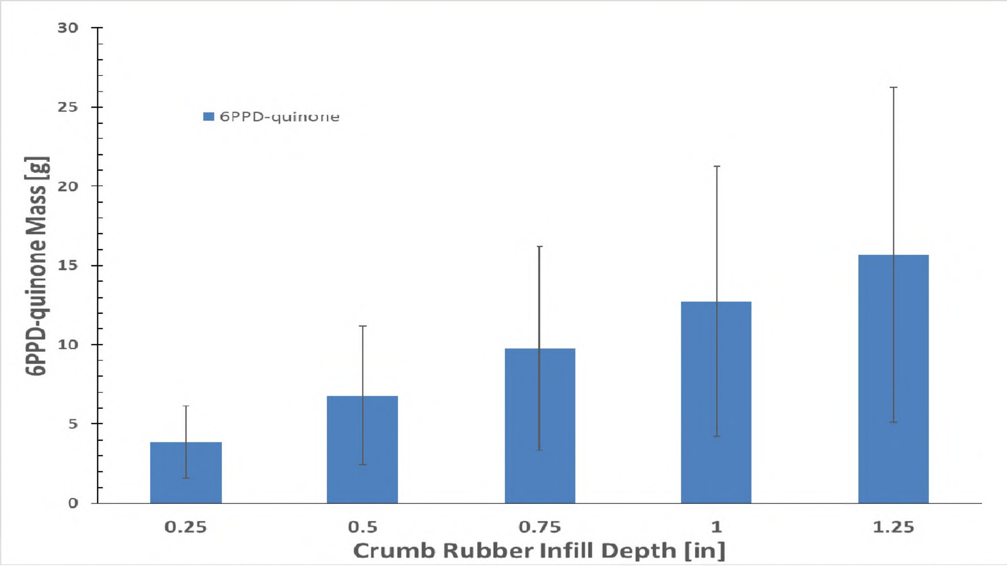

Figure 4. Estimated total mass of 6PPD-quinone discharged to the Puyallup River during the July 29-30 spill event. Estimates were based on measured 6PPD-quinone concentrations and compositions of provided crumb rubber samples and the 617 square yards of field turf reported discharged to the river by Cherry (2020). Error bars reflect observed standard deviations of 6PPD-quinone composition in crumb rubber infill materials.

Comparison of the above mass estimates with the 0.095 µg/L LC50 value for coho salmon would indicate that the mass of 6PPD-quinone discharged by the spill event has the potential to bring 10,700,000-43,700,000 gallons of water to concentrations where acute mortality would be expected to occur in coho salmon (Figure 5).

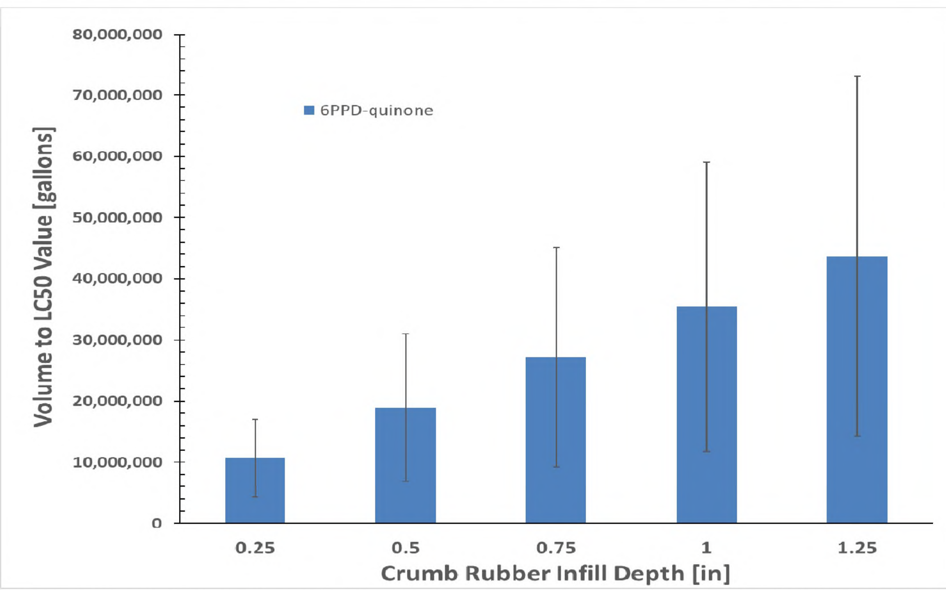

Figure 5. Estimated maximum volumes of water brought to LC50 values for 6PPD-quinone released to the Puyallup River during the July 29-30 spill event based on mass discharges. Error bars reflect observed standard deviations of 6PPD-quinone composition in crumb rubber infill materials.

While these water volumes are large, during July 29-30, 2020, approximately 600-800 cubic feet per second (cfs) of water flow was measured in the Puyallup River (USGS gage 1209200 “Puyallup River near Electron). An average discharge of 700 cfs represents ~450 million gallons per day of river flow. Because this concentration is ~17-fold higher than the water volume capable of being brought to near lethal concentrations (at 0.75” infill depth), it is unlikely that the entire Puyallup river volume would have experienced acutely lethal concentrations during the Electron Dam crumb rubber spill event. Instead, potential exposures to lethal concentrations might be more probable for those waters in very close proximity to spilled crumb rubber and turf, such as areas near contaminated sediments or within gravels, side channels with slower flows, or more stagnant areas where reduced water volumes allow higher concentrations to leach from spilled crumb rubber and turf materials before dilution with cleaner waters occurs.

Appendix

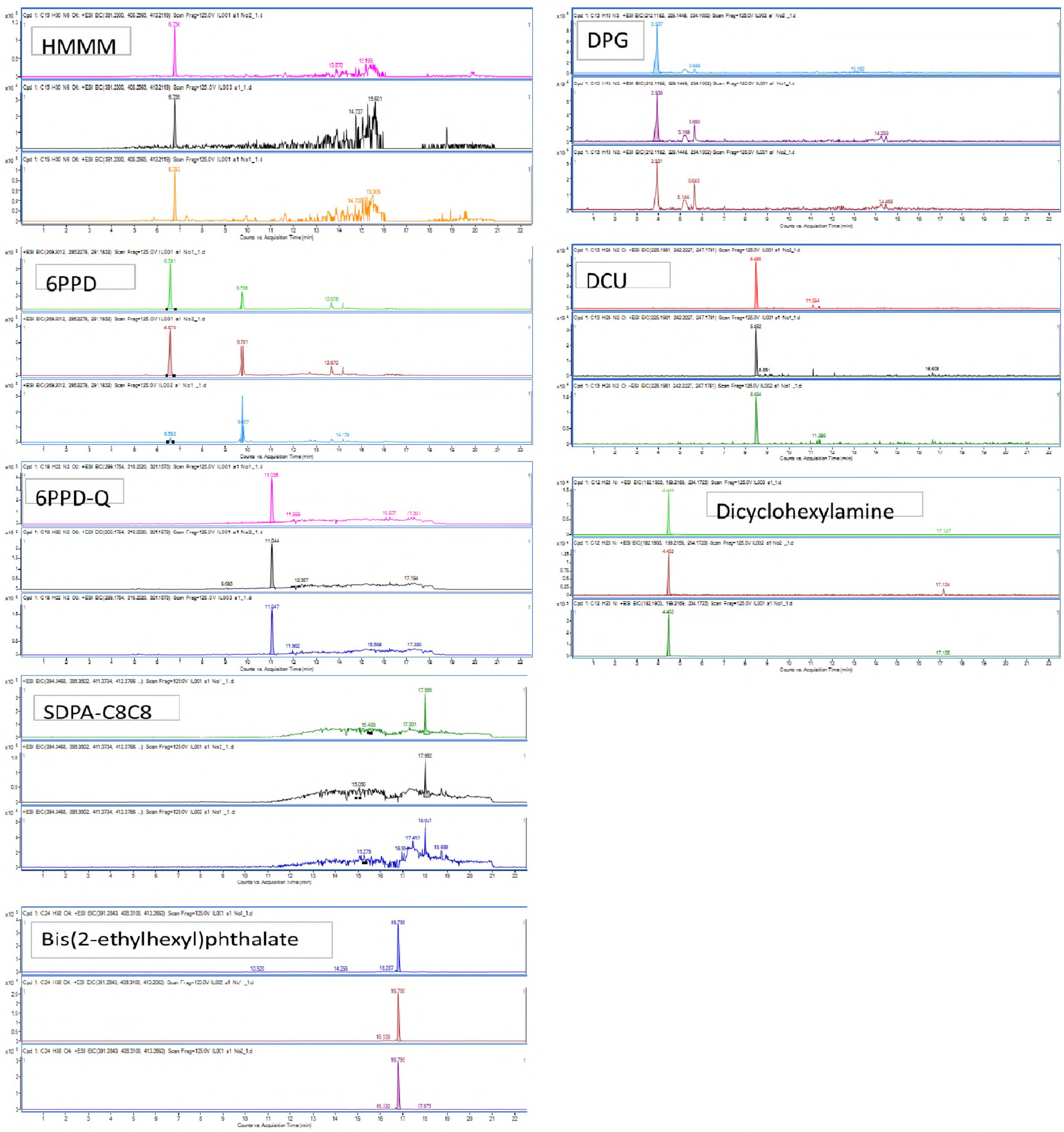

Figure 6. Chromatograms and identifications of tire rubber derived contaminants in intake samples IL001a1, IL002a1, and IL003a1, as identified by QTOF-HRMS analysis.

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Draft Sampling Plan: February 26, 2021
(updated Feb 3, 2022)

Project Objective and Background

The Washington State Attorney General's Office Environmental Protection Division Criminal Section is seeking to collect samples of crumb rubber from rolls of artificial turf located at Washington Rock King Creek Quarry as part of its investigation into potential violations of Washington Law to have occurred at the Electron Dam in the summer of 2020. Weather permitting, Washington Department of Fish and Wildlife Sergeant Ken Balazs will lead a detail of WDFW officers and other law enforcement/EPA personnel to the location of the King Creek Quarry, located outside of Orton. The sampling team will meet at a location and discuss the sampling plan and procedure prior to travelling to the sampling site. At the site, Sgt. Balazs or EPA personnel will obtain at least triplicate samples (defined as "from the same roll") of crumb rubber and turf material from five (5) or more rolls of artificial turf that have been stored at the property, representing at least 15 independent samples. Once the samples have been obtained, Sgt. Balazs will ensure the appropriate handling and chain of custody procedures are followed to ensure the samples remain secure and uncontaminated until they are delivered to the University of Washington Center for Urban Waters for analysis.

Pre-sampling Site Visit

Prior to the sampling event, a site visit should be conducted at the King Creek Quarry to visually examine the rolls of artificial turf and assess their current weight and size. The site visit should be conducted at the location circled in Exhibit 1. While at the site, photographs need to be taken to note the condition and variances in the turf materials that may need to be accounted for prior to sampling. Additionally, an assessment should be made regarding viability of moving the rolls of turf manually or if heavy machinery will be necessary to move the materials to extract unweathered samples from deeper in the pile.

Sampling Provisions

Sampling should occur at the location circled in the photograph attached as Attachment A. At the sampling site, five (5) rolls of artificial turf should be selected based on their location in the artificial turf pile. At least two rolls from each of the top and middle layers should be selected. See Attachment B, the attached diagram for example sampling locations in the rolls. Additionally, different colors of artificial turf, (e.g. both red and green), should be selected if these are broadly representative of the pile composition. Once the artificial turf rolls have been identified for sampling, photographs are to be taken of the roll where it was found in the pile with an exhibit marker, marking each roll with a number, beginning with 1. Once initial photographs are taken identifying the roll, the roll will be moved to the ground. The team members who helped moved the roll will be wearing face masks and work gloves. The names of the sampling team that moved the roll will be documented. Any equipment used when the rolls were moved shall be documented. The roll should be placed on visqueen sheeting that have been placed on dry or protected ground if possible. The rolls should remain dry and protected from the elements to the best possible extent.

Once the artificial turf roll is on the ground, an additional photograph will be taken with the corresponding exhibit marker. The roll will then be unrolled. Once the artificial turf is now flat and completely unrolled, at least three (3) samples of crumb rubber are to be collected from three different locations from the artificial turf, by collecting samples about 50-75% of the way into the roll. For each roll sampled, cut out and collect one 6" by 6" sample of turf material adjacent to the center crumb rubber sample location. The sampling locations on the artificial turf will be documented and photographs shall be taken identifying each sampling location with the corresponding exhibit number and a letters to identify the sampling location. For example, the turf sample from the first roll will be identified as exhibit 1T to indicate it is the turf sample, the crumb samples will be labelled 1CR-A, 1 CR-B, and 1CR-C. To prepare for sampling, sample jars corresponding to each sample location should be pre-labeled, without opening the jars. Care must be taken not to touch, step or walk on the sampling locations, or contaminate samples with exterior/foreign materials. Please note any relevant observations or issues related to sample collection on the chain of custody materials as needed. Please collect any QA/QC samples (see table below) in an identical manner, with pre-labeled and photographed sample jars.

Table 1: Recommended labeling format and sample designator for each of the collected sample types.

Sample Type and Label	**Turf**	**Crumb Rubber #1**	**Crumb Rubber #2**	**Crumb Rubber #3**
Roll 1	1T	1CR-A	1CR-B	1CR-C
Roll 2	2T	2CR-A	2CR-B	2CR-C
Roll 3	3T	3CR-A	3CR-B	3CR-C
Roll 4	4T	4CR-A	4CR-B	4CR-C
Roll 5	5T	5CR-A	5CR-B	5CR-C
Additional rolls, as able	#T	#CR-A	#CR-B	#CR-C
QA/QC samples				
One set of used nitrile sampling gloves	NT-Glove			
One large sample jar filled with a sample of new, unused visqueen sheeting	VSQ-Sheet			

Once the sampling locations are properly documented, Sgt. Balazs will then use a clean stainless steel garden hand trowel or spoon to remove at least 50 grams of crumb rubber from the artificial turf and place the rubber crumb into the pre-labeled glass sample jars. A digital scale will provided to ensure a sufficient amount of crumb rubber is obtained. If crumb rubber collection is easy, the jars should be filled as much as practicable. For the single turf sample collected for each roll of artificial turf, cut out the turf sample with the rubber crumb intact and then place both the turf and rubber crumb in the same, large sized sampling jar.

Sgt. Balazs is to wear clean nitrile gloves for each sample collection.

Once a sufficient sample is collected, the glass jar label will be re-checked against the roll and sampling location to insure proper labeling and then sealed with red evidence tape, initialed by Sgt. Balazs. The sealed sample jar with the appropriate labelling needs to be photographed at each sampling location. Sealed jars with the collected samples must be kept under the custody of a single member of the sampling team and their name shall be documented in the paperwork. Prior to removing nitrile gloves, the stainless steel sampling instrument should be wiped clean with lab wipes between each sample to remove any adhered material or residual moisture. The sampling implement should be clean and dry between sample collections.

This process above is to be repeated three (3) times for each roll of the five (5) artificial turf rolls, for a total of at least 15 discrete crumb rubber samples and 5 turf material samples. Additional samples can be collected from various locations if the sampling team feels they are merited or would provide value (for example, many turf colors, or evidence of multiple turf types, or further sampling of other locations within the site). Worksheets will be provided for each roll selected.

Two (2) additional QA/QC samples (nitrile gloves and visqueen sheeting) will also be collected and included in the sampling effort. Approximately midway through the sampling day, one set of Sgt. Balazs' used nitrile gloves will be placed in an additional glass evidence jar for subsequent analysis and documented. At this time, a sample (fill the large jar) of clean, unused visqueen sheeting should also be collected and documented.

Once all of the sampling is completed, Sgt. Balazs will take possession of the sealed jars containing the samples and check the jars into evidence at the WDFW Office.

Equipment Needed

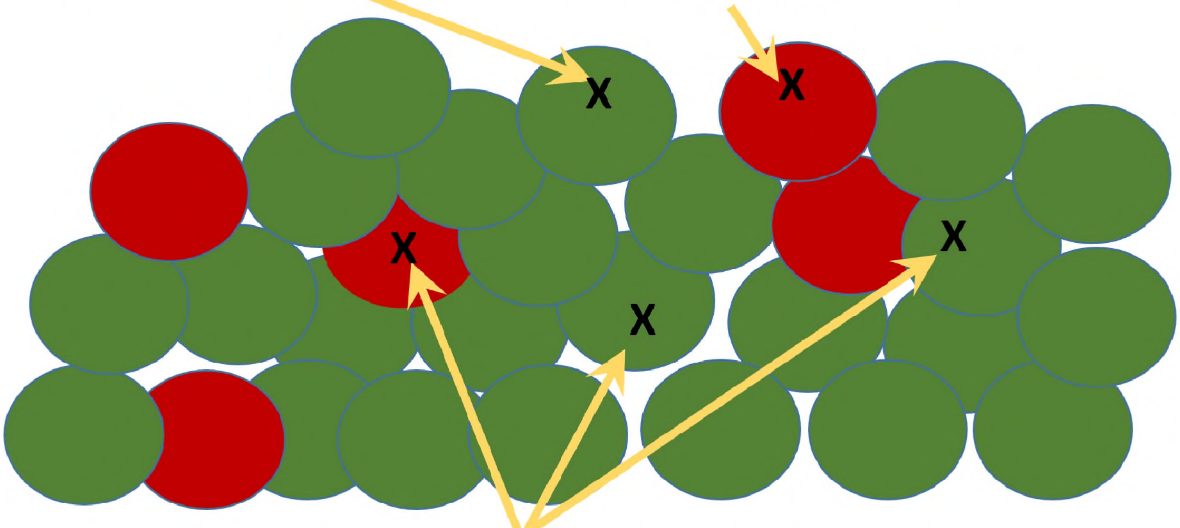
1. Facial Coverings, N95 masks preferred
2. Work Gloves
3. Nitrile Gloves for handling of Samples
4. Portable Electronic Scale to ensure sufficient sample amounts
5. Digital camera
6. 20-40 Sealable Glass Jars with labels to preserve samples and gloves for further sampling
7. Red evidence tape to seal jars
8. Protective Eyewear
9. Pens or other permanent markers for sample labeling
10. Clean exacto Knives, pocket knife or other cutting equipment
11. Hand held stainless steel garden trowel or spoon to collect crumb rubber from artificial turf roll.
12. Wipes to wipe off stainless steel sampling equipment prior to each use.
13. Visqueen sheeting to protect roll in roll sampling area
14. Straps/crowbars to help move the artificial turf rolls
15. Trash bags for any used sheeting or discarded materials.

Attachment A

Attachment B1

Top (weathered) roll, green.
Collect 3 samples from top roll,
About 50-75% of the way into the
Roll.

Top (weathered) roll, different turf color.
Collect 3 equivalent samples from top roll,
about 50-75% of the way into the roll.
Exclude if not present.



Deep (unweathered) rolls, green and colored. Sample well, these best reflect the pile.
Collect 3 samples from each of three rolls, reflecting the "average" observed turf color and composition (e.g. 2 green, one colored; or 3 green if mostly green)
Sample about 75% of the way into the roll, trying to collect samples that are protected from the elements. If unfeasible, collect as deep as possible, and find rolls protected from the weather. Try to sample roughly the midpoint of the piles.

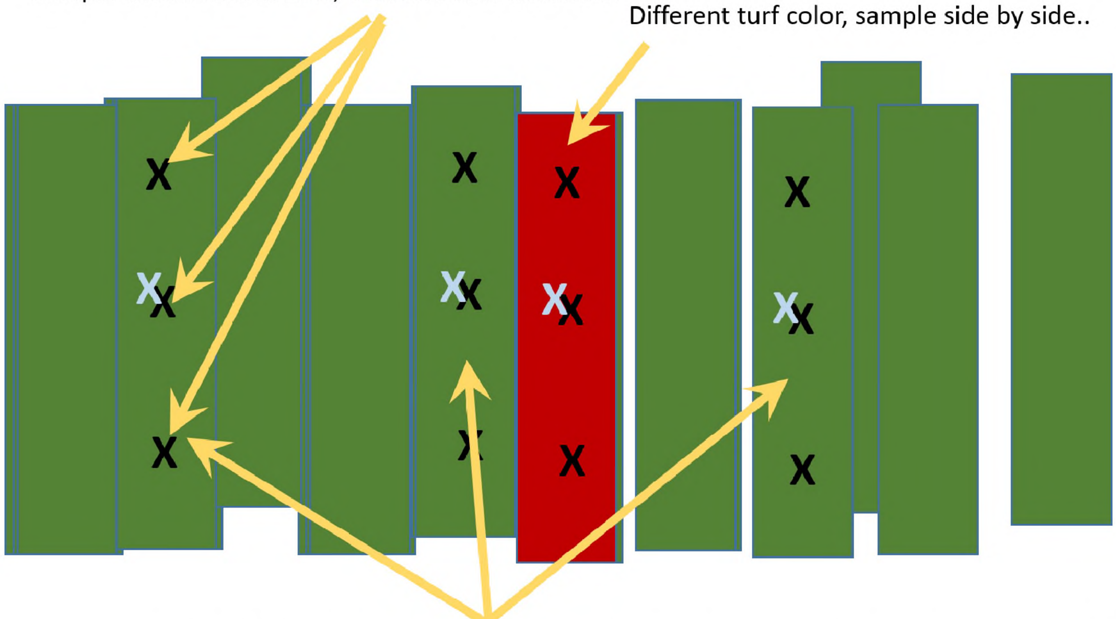
Side View, sampling plan. Describes representative rolls to collect.

Exhibit B2

X = Crumb rubber sample location
X = Turf material sample location

Sample within rolls at 25%, 50% and 75% distances.

Different turf color, sample side by side..



Deep (unweathered) rolls, green and colored. Space samples apart, from rolls at least 8-10’ apart. Space the three midpoint rolls out through the source pile area (rolls should not be next to each other). Weathered (top rolls) can be collected as convenient.

Top View, sampling plan. Where to collect samples from.

Washington Rock King Creek Quarry Personnel Roster:

Name, Organization, Contact Phone Number	Sampling Duties
1. Sgt. Ken Balazs, WDFW, ________________	Primary Sampler and Custodian
2. ______________________________	On-scene evidence custodian
3. ______________________________	Photographer
4 ______________________________	Protocol Management (Fill out checklist)
5. ______________________________	Identification of Roll and Sampling locations
6. ______________________________	Liaison to Washington Rock Quarry
7. ______________________________	
8. ______________________________	
9. ______________________________	
10. ______________________________	
11. ______________________________	

Meeting time and location:

9:00 a.m.

Sampling Checklist
Artificial Turf Roll 1

☐ Current weather (state if cloudy, drizzling, raining) ____________________

☐ The roll is from the location depicted in the satellite image attached as Attachment A

☐ Photograph of the Roll where it was found in pile with Exhibit 1 card

☐ Team members who moved the Roll

- ______________________________________
- ______________________________________
- ______________________________________
- ______________________________________

☐ Equipment used to move Roll

- ______________________________________
- ______________________________________
- ______________________________________

☐ Roll Placed on Visqueen sheeting in sample collection area to keep sample collection area as clean and dry as possible.

☐ Photograph of the Roll with Exhibit A printout

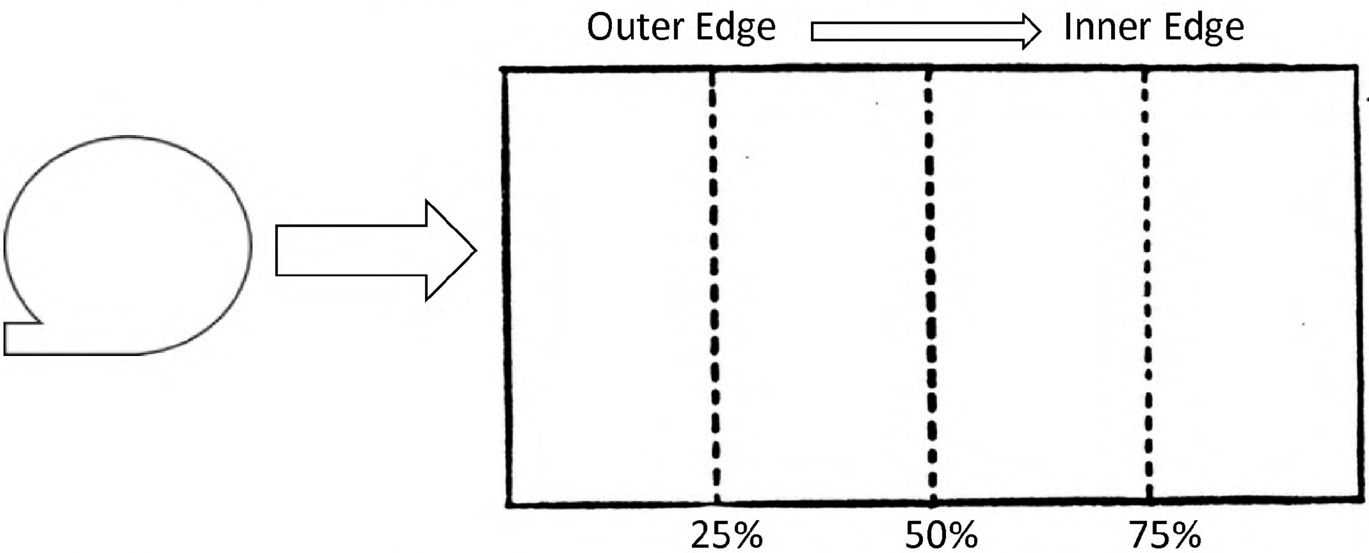
☐ Unroll the artificial turf and take another photograph with Exhibit A card

☐ Team members who unrolled the turf roll

- _____________________________
- _____________________________
- _____________________________
- _____________________________

☐ Documentation of Sampling locations

- Mark on diagram with the sampling marker- 1T, 1CR-A, 1CR-B, or 1CR-C. 1T is the location where a turf sample is taken. Samples should be taken near the dashed lines are shown in the diagram.



☐ Photograph Sampling Locations on the turf with Exhibit 1T, 1CR-A, 1CR-B, or 1CR-C

☐ Fill out label on capped glass sampling jar, including the sampling location (e.g. 1CR-A, 1CR-B, etc)

☐ Place glass jar on a digital scale and tare the scale to ensure it is ready to weigh crumb rubber

☐ Sgt. Balazs to put on new pair of nitrile gloves

☐ Uncap jar, then use clean stainless steel handheld trowel or spoon to scoop crumb rubber into glass jar if crumb rubber is being collected. For turf samples, cut a 6” by 6” piece of turf out from the center location, and place turf sample and any crumb rubber infill material into large sized sample jar.

☐ Carefully protect the jar cap interior from dirt and foreign material during sampling. Cap glass jar immediately upon sample collection.

☐ Weight of rubber crumb samples (turf material samples do not need to be weighed).

- 1 CR-A =__________ grams
- 1 CR-B =__________ grams
- 1 CR-C =__________ grams

☐ Check jar is fully closed, seal with red evidence tape, recheck label correctness, and initial

☐ Provide to Evidence Custodian, Name ____________________